Cell uptake survey of functionalized Graphene for Near-Infrared Mediated tumor Hyperthermia

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The exciting advances in the preparation of nanosystems with applications in the medical field have lead to new challenges in the design of smart materials capable of meeting the clinical demands. Therefore, the application of nanotechnology for treatment, diagnosis, monitoring and control of biological systems (recently denominated as nanomedicine) has been declared as one of the most promising fields of research over the last decade. Mainly after finding that the introduction of nanosystems into living cells is possible by shuttling various cargoes across cellular membranes, without producing cytotoxicity.

Nanoparticles have been proposed for locally releasing highly toxic drugs directly into tumors, while reducing the unwanted side effects of aggressive treatments, and to target tumors using the intrinsic capacities of different nanomaterials applied as new treatments. For example, their capacity to induce localized heating within tumors is being exhaustively explored. Approaches to nanoparticle-mediated thermal therapy include absorption of infrared light, radio frequency ablation, and magnetically-induced heating [1].

Among carbon based materials, following close and taking over carbon nanotubes, graphene represents one of the most promising “nanoparticle” of the last few years. More specifically, graphene oxide (GO), it is a small two-dimensional shape nanoparticle [2], that offers a new class of solution-dispersible polyaromatic platform for performing chemistry and which aspect ratio make it incomparable to any other previously suggested one. Besides all its properties, its low cost, large production scale and easy-processing, makes this nanomaterial a promising nanoparticle for medical application were a large scale production is needed. Its unique structure, with all atoms exposed on its surface, has an ultra-high surface area available for efficient loading of aromatic drug molecules, useful for applications in drug delivery. Also, its strong NIR optical absorption ability 700-1100 nm (“*therapeutical window*”, where it is a non invasive, harmless and skin penetrating irradiation) range is particularly attractive for the induction of cells hyperthermia in tumor treatments as a minimally invasive alternative to surgery (Photothermal therapy). This therapy is based on the transfer of energy produced during the irradiation of a material, after being internalized by a cell, generating vibrational energy, thus generating heat sufficient for cell destruction.[3]

With the purpose of understanding the biological response of cells to this graphene uptake, a complete in vitro biocompatibility and internalizing kinetics study of GO sheets has been performed in a survey of different kind of cells: osteoblast, preosteoblasts, fibroblast, and macrophages.

GO nanosheets of c.a. 100 nm have been obtained from exfoliation of high purity graphite by a modified Hummers method [4] see Fig. 1, and functionalized in its surface with non-toxic and non-immunogenic polymers to avoid the intercession with cellular functions or target immunogenicities and to decrease aggregation. Atomic force microscopy (AFM) and transmission electron microscopy (TEM) were used to measure the thickness and size of the synthesized GO nanosheets. Moreover, the GO particles have been labeled with a fluorescent marker (fluorescein FITC) as this is an important requisite

for their in vitro studies with cells both for the application of Confocal Microscopy and Flow Cytometry to follow uptake and possible degradation within cells.

Different cell types have been cultured for several incubation periods in the presence of 0.075 mg/mL GO solutions dispersed in supplemented Dulbecco´s Modified Eagle Medium (DMEM). GO-FITC uptake kinetics, cell morphology, viability and proliferation, lactate dehydrogenase release were evaluated in vitro by flow cytometry, and confocal microscopy. Incorporation assays of GO-FITC in the presence of Trypan Blue, in order to quench the extracellular fluorescence, demonstrate the intracellular location of this material.

Results show that cell viability measured by propidium iodide exclusion is always above 95% for all the cells type studied after 30, 60 and 150 min and 1 day, suggesting that there is not induction of apoptosis or necrosis by the nanomaterial. As an important discovery, GO uptake kinetics revealed several differences in uptake speed as a function of the type of cell involved. It has been demonstrated that, for example in the case of osteoblast like cells (see Fig.2), uptake kinetics are faster suggesting that GO can more easily penetrate the cell membrane without resulting in greater cell membrane damage. Our studies have clearly demonstrated that the uptake of GO nanosheets by the cells plays an extremely important role in how efficient and controlled the treatments would be, and has given light to the future definition of timing parameters and selective uptake control by certain type of cells in order to achieve an effective therapy.

References

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Figures

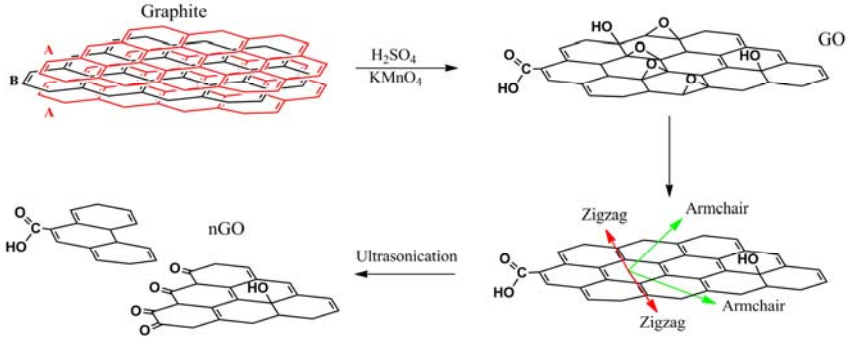

Figure 1. Scheme of GO exfoliation

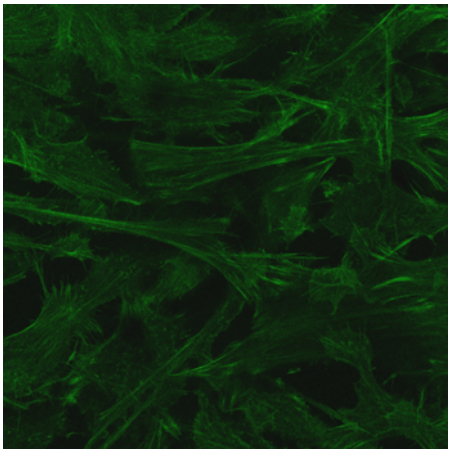

Figure 2.Confocal Microscopy image of osteoblast-like cells (MC3T3). Green fluorescence due to fluorescein labeled GO excitation.